

NOTE ON APPARENT APOGAMY IN *PTERYGODIUM*
NEWDIGATAE.

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(Plates XLII, XLIII.)

Pterygodium Newdigatae is represented by the normal open-flowered species and a cleistogamous variety, which differs greatly from the type in perianth and column. The forms which were regarded by Dr. Bolus as constituting the normal species are represented by three individuals now in the Bolus Herbarium and one in the Government Herbarium, Cape Town. These were collected by Miss Newdigate near Forest Hill, in the Knysna district, in 1894. The cleistogamous form occurs in some abundance, growing among grass and bush in sandy and stony localities in the districts of George, Knysna, Humansdorp, and Albany ; but, so far as I am aware, no further specimens of the open-flowered form have been found of late years in spite of careful search.

Instances of cleistogamy, though rare, are not wanting among Orchids, but the occurrence of strikingly distinct chasmogamous and cleistogamous forms seems unknown in the case of any other Orchid species.

Dr. Bolus, in his monograph on *Orchids of South Africa*, concludes his description of the cleistogamous form as follows : " No open or punctured flower has, as yet, been observed ; no pollen has in any case been found upon the stigma ; yet the ovaries have swollen, producing abundance of seed, and Dr. Schoenland, who examined them, found the seeds to contain a perfect embryo. The question remains, How is the fertilisation effected ? This, with some other points in respect of this remarkable species, must await for its elucidation a supply of fresh material, which Dr. Schoenland, who has kindly assisted me in this matter, hopes to obtain during the ensuing season."

Rolfe, in his account of the species in *Flora Capensis* observes : " The column is very variable in the normal cleistogamic form, three conditions being figured by Bolus. Schlechter makes it a species, under the name of *Pterygodium cleistogamum*, but it is clearly only a peloriate, self-fertilising form of *Pterygodium Newdigatae*."

As much interest attaches to the method of reproduction and mode of fertilisation in this Orchid, I undertook an investigation into these processes.

Although this investigation is still far from complete, it seemed advisable to publish a preliminary account of certain of the facts observed.

Material, of the cleistogamous form only, was collected in the Knysna district on several different occasions. The spikes have usually from three to seven flowers, but single-flowered spikes are infrequently found. The largest specimen examined was nine-flowered. In colour the perianths vary from pale green to yellow flushed with orange. Young specimens are readily confused with buds of *Pterygodium catholicum*, often found growing in the same situations. As the buds swell, the difference between the two species becomes more apparent, but even in the early stages the powerful almond scent of *Pterygodium Newdigatae* is very distinctive.

I have examined some hundreds of flowers and noted considerable variation in the inner perianth lobes. A large percentage of specimens received from Knysna on November 9, 1914, had one or occasionally both side petals well developed, and of a deeper yellow colour and more delicate texture than other floral parts (Figs. 2-5). A thickening at the base of the labellum was of fairly frequent occurrence (Figs. 7-9). The closest approach to the perianth of the chasmogamous form was observed in a single-flowered specimen received on November 20 (Figs. 6, 10, 11). The parts, which were at first united, sprang apart during the handling of the specimen, disclosing a column of abnormal form (Figs. 12, 13).

The column in the cleistogamous variety is exceedingly variable. Dr. Bolus figured three distinct forms; I have examined some 500 flowers and noted the following varieties:

(a) Rostellum arms 2, undivided; column occasionally symmetrical (Fig. 14), but more frequently showing a difference in length, breadth, or curvature of the arms (307 examples).

(b) Rostellum arms 2, variously lobed or divided. The lobing may affect the sterile tissue only, or the fertile tips, or both (Figs. 15, 16) (75 examples).

(c) Rostellum arms 4, undivided. As a rule the two pairs of arms are somewhat widely separated (Figs. 17-20) (48 examples).

(d) Rostellum arms 4, variously lobed or divided (Fig. 21) (17 examples).

(e) Rostellum arms 3, contorted or slightly lobed (Fig. 22) (12 examples).

(f) Extreme abnormalities (Figs. 23-25) (22 examples).

The drawings give some idea of the enormous variation of the column met with in this Orchid, which is in striking contrast with the conservative character of the essential organs in allied species and throughout the entire order.

The two-armed form is by far the commonest, but does not seem to have been represented in the material examined by Dr. Bolus. Further development and branching of this form may have given rise to the variations noted above.

Although a single type of column is usually constant for any one spike, yet individuals were observed in which the number and shape of the rostellum arms of the different flowers showed considerable variation.

The strong perfume of the cleistogamous flower is possibly a relic of the time when insect pollination was essential for seed production. The zygomorphy of the normal flower is more or less completely lost in the cleistogamous form, but resupination of the ovary occurs as usual.

The small gland-like pollen masses (?) remain permanently embedded in the tips of the rostellary arms (Fig. 26). The supposition that the pollen grains germinate *in situ* proved to be incorrect. Freehand sections through the base of the column of flowers of various ages showed no trace of pollen tubes, though these are readily demonstrated in the case of other Orchid flowers.

Material, collected at different times, was fixed in chromacetic acid and microtomed. The youngest buds available had ovaries from 3-4 mm. in length. The tips of the rostellary arms contained compact sporogenous tissue (?), composed of somewhat elongated cells with dense protoplasm and well-defined nuclei (Fig. 27). No indication of nuclear division was observed. The tissue appeared to be in the mother-cell stage. Ovules of the same flower showed no trace of integuments. The single hypodermal cell enlarges and functions directly as the megaspore-mother-cell (Fig. 28).

Somewhat older flowers exhibited no further development of sporogenous tissue (?) in the rostellary arms (Fig. 29). The megaspore-mother-cells, however, appeared for the most part in synapsis (Fig. 30). This condition evidently lasts for some time, as not only was it present in material collected on several different occasions, but was also found to occur in inverted ovules with fairly well developed integuments (Figs. 31-33). In no case was there any trace of pollen tubes in the ovary, in the tissues of the column, or in the rostellum arms. The statement is often made that Orchid ovules do not develop far unless pollination has occurred. It is worthy of note that in the species under discussion ovular development is apparently completed in the absence of fertile pollen.

Unfortunately several gaps occur in my material, the most serious being from October 19 to November 8; thus, further stages in the development of the megaspore-mother-cell and embryo sac are missing. Ovules of flowers received on November 8 contained young embryos, while much of the material received two weeks later had the embryos fully formed. Microtome sections through the rostellum arms of flowers with conspicuously swollen ovaries showed no appreciable change in the archesporial (?) tissue. Fig. 34 is a drawing of isolated cells from the disorganising sporogenous (?) tissue of a flower, the seeds of which had already been shed.

Sterility of the anthers has been shown in certain genera to be associated with apogamous development of the embryo. It seems reasonable to conclude that we are here also dealing with a case of apogamy.

Various stages in the development of the rudimentary embryos were observed (Figs. 35-39). While the seeds are immature, the intermediate layers of the ovarian wall, the cells of the placenta, and the suspensors are packed with starch. This disappears, and the suspensors begin to disorganise before the seeds are shed. Suspensors are often seen reaching almost to the apex of the integument, but they do not project from the micropyle and penetrate the placental tissue as has been described in many Orchid species.

Polyembryony is of very common occurrence in *Pterygodium Newdigatae*. The two embryos are often of approximately equal size, though one may be markedly smaller than the other (Figs 40-42). In order to get some idea of the frequency of polyembryony in this species, ripe seeds were removed from various parts of mature ovaries and placed in alcohol to allow them to separate. The average of a number of counts showed that as many as 12 per cent. of the seeds of an ovary may exhibit polyembryony. Three embryos may occur within one seed coat, but this condition is not frequent (Fig. 43). It is possible that cases of two mother-cells in a single ovule, as figured in 44, may account for some of the additional embryos. Again, instances were noted of an apparent segmentation of the embryonal mass, but the detailed explanation of the origin of polyembryony in this Orchid must await the examination of more suitable material.

The dehiscence of the clavate fruit is unlike any of those mentioned by Pfitzer in his discussion of this order in *Die natürlichen Pflanzenfamilien*, Part II, sect. vi. The capsule splits by three longitudinal fissures into three broad and one narrow valve, which remain connected at base and apex. The narrow valve consists of the midrib of the carpel below the odd sepal; the two broad valves lying on either side are each composed of two united half carpels together with a carpellary midrib (Figs. 45, 46).

An attempt is being made to grow this Orchid from seed, in the hope that some light may be thrown upon the relationship existing between the open and closed flowered forms. Seeds dusted over the outer surface of unglazed porcelain cylinders kept constantly moist, were found to be appreciably swollen less than three weeks later. The seed coats were split, and delicate unicellular hairs had emerged from certain of the superficial cells of the embryos (Fig. 47). In older seedlings these hairs had increased considerably in length, some being three times as long as the testa. Where two embryos occurred within a single integument, both, as a rule, showed signs of germination (Fig. 48). The seeds were collected in November, 1914, but seed which had been kept dry for considerably over a year failed to germinate. Several distinct species of fungi occur in profusion among the germinating seeds (Fig. 49). Whether the seedlings will complete their development, thus indicating the presence of the necessary mycorrhizal fungus, remains to be seen.

It seems probable that there is some relationship between the extraordinary polymorphism of this species and its apparent apogamy.

The exact nature of the nuclear changes in the female elements will be investigated after a complete series of the various stages has been obtained.

The above investigation was commenced with the assumption that the gland-like bodies at the tips of the rostellum arms are reduced anther cells. An examination of the closely allied species, *Pterygodium catholicum*, has suggested the possibility that the masses of tissue in question may represent the rostellary glands. On dissecting away the anther from young flowers of *Pt. catholicum*, the appearance presented by the rostellary arms is strikingly like that of the two-armed form of *Pt. Newdigatae*. A microscopic examination of the gland-like tissues in each case reveals a further similarity. If this supposition should prove to be correct, then we have in the cleistogamous form of *Pt. Newdigatae* complete suppression of the fertile anther together with the lip appendage, which is considered by some as representing the posterior stamen of the inner whorl.

EXPLANATION OF PLATES XLII, XLIII.

FIG.

1. Petal of peloriate cleistogamous flower.
2. Odd sepal coherent with side petals, one of which is somewhat petaloid.
- 3-5. Side petals showing various stages of development.
6. Side petal of single-flowered spike showing striking similarity with petal of chasmogamous species.
7. Lip with tooth-like appendage on one margin.
- 8, 9. Lip with basal thickening.
10. Well developed lip of single-flowered spike.
11. Flower with well-developed petals. Details of floral parts figured in 6, 10, 12 and 13.
- 12, 13. Back and front view of column of the flower figured in 11.
14. Rostellum arms 2, undivided.
15. Rostellum arms 2, one arm lobed.
16. Rostellum arms 2, both lobed.
17. Rostellum arms 4, approximating.
18. Rostellum arms 4, the two pairs widely separated. One arm shows two masses of sporogenous (?) tissue at its tip.
- 19, 20. Rostellum arms 4, the two lowest sterile.
21. Rostellum arms 4, the two lowest divided.
22. Rostellum arms 3, in one of which the sporogenous (?) tissue is lobed.
- 23-25. Extreme abnormalities of column.
26. Enlarged tip of rostellum arm showing embedded mass of sporogenous (?) tissue,
27. Section through sporogenous (?) tissue of very young flower.
28. Ovule from the youngest ovary examined, showing hypodermal megaspore-mother-cell.
29. Section through sporogenous (?) tissue of older flower.
30. Young ovule: nucleus of mother-cell in synapsis.
- 31-33. Older ovules with mother-cell still in synapsis.
34. Isolated cells of sporogenous (?) tissue from flower with dehiscent ovary.
- 35, 36. Stages in development of embryo.
- 37, 38. Ovules with suspensors almost reaching micropyle.
39. Embryo in transverse section.
- 40-43. Polyembryony.
44. Ovule with two mother-cells.
45. Dehiscent capsule showing narrow valve below odd sepal.
46. Diagram to show method of capsular dehiscence.
47. Early stage in germination of seed.
48. Two embryos germinating within one seed coat.
49. Fungi present among germinating seeds.